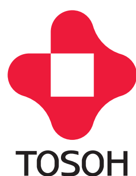

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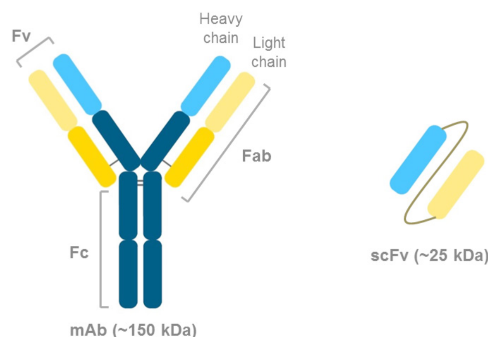
Application Note

CAPTURING OF A scFv FROM *E.COLI* USING TOYOPEARL® AF-rPROTEIN L-650F

An application example for the general method set-up and optimization

scFv CAPTURING

Single chain variable fragments (scFv) are antibody derived molecules. The variable part of the light chain and the heavy chain of a mAb are connected via a peptide linker. The molecular weight of the whole construct is approximately 30 kDa. ScFvs are apt for being produced in E.coli, since they lack the glycosylation site of a full-length IgG. Other advantages over a full-length IgG include rapid target access and good tissue penetration. However, renal clearance delimits serum half-life to hours instead of days, compared to typical serum half-lives of mAbs. In some therapeutic strategies, this may be an advantage. Hence, straight-forward and efficient capturing solutions similar to Protein A for mAbs may pave the way for the future success of this class of molecules.



Herein we describe general conditions for capturing of an exemplary scFv from E.coli with TOYOPEARL AF-rProtein L-650F. Further, host cell protein (HCP) removal was optimized in a parallel chromatographic approach.

LAB-SCALE EXPERIMENT

The κ light chain of mAbs, Fabs and scFv binds to Protein L at neutral pH and physiological conductivity. HCPs flow through the column at the applied conditions. The bound product can then be recovered at pH 2.0-3.5, depending on the particular format and molecule. As a rule of thumb, larger molecules can be eluted at comparatively higher pH. Most methods can be set-up based on these general guidelines. In detail, the herein presented experiments use 100 mM sodium phosphate buffer, pH 6.5 for column equilibration. 15 column volumes (CV) of a periplasmic scFv feedstream were loaded onto the 6.6 mm ID x 6.7 cm L column packed with TOYOPEARL AF-rProtein L-650F. The column was washed with equilibration buffer and the scFv was recovered in 100 mM glycine/HCl, pH 2.0. The method was conducted at 300 cm/h except for column loading, which was conducted at 3 min residence time.

Figure 1 shows the lab-scale capturing of scFv. 15 CV of the feedstream were loaded on the column at 3 min residence time. HCP flow through the column during loading and washing was done with 0.1 M sodium phosphate, pH 6.5. As a result the scFv eluted as a sharp peak in 100 mM glycine/HCl, pH 2.0.

scFV LAB SCALE CAPTURING

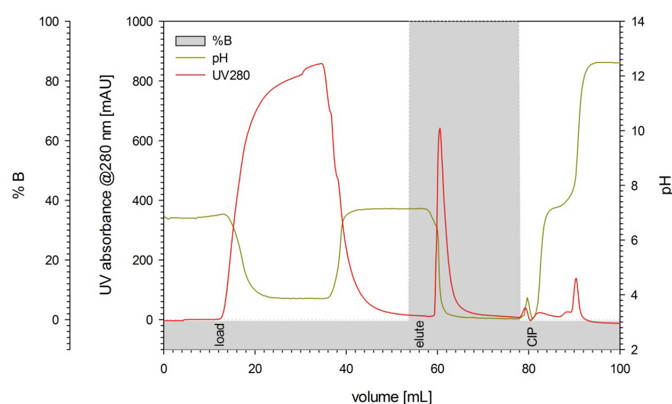


Figure 1

THE DIFFERENT PROCESS STEPS AND CORRESPONDING BUFFERS OF PROTEIN L CHROMATOGRAPHY

Step	CV	Composition
Equilibration	10	100 mM NaP, pH 6.5
Load	5	feedstream from E.coli
Wash 1	5	100 mM NaP, pH 6.5
Wash 2	5	variable
Wash 3	5	100 mM NaP, pH 6.5
Elute	5	100 mM glycine/HCl pH 2.0
CIP	1	50 mM NaOH
Reequilibration	10	100 mM NaP, pH 6.5

Table 1

WASH SOLUTIONS APPLIED IN WASH 2, THE INTERMEDIATE POST-LOAD WASHING STEP

Buffer	Additive	Additive concentration
100 mM NaP, pH 6.5 (reference)	-	-
100 mM NaP, pH 6.5	arginine	1 M, 2 M
100 mM NaP, pH 6.5	guanidinium hydro-chloride	1 M, 2 M
100 mM NaP, pH 6.5	sodium chloride	0.5 M
100 mM Na acetate, pH 6.5	sodium chloride	0.15 M, 0.5 M

Table 2

METHOD OPTIMIZATION

Screening of chromatographic conditions can significantly improve performance of the corresponding process step. In order to reduce sample consumption and to save time, the method was down-scaled to 200 µl RoboColumn® format (P/N 0045066) and the feed loading was reduced to 5 CV. Parallel chromatography was conducted using a Tecan Freedom Evo® 150 liquid handling station equipped with a chromatography station and a UV plate reader.

In the current study, different intermediate wash solutions were tested with regards to their potential to improve HCP removal compared to the use of the equilibration buffer for post-load washing. A detailed list of the applied buffers and solutions can be found in table 1. Flow rates were adopted from lab-scale chromatography. The different solutions applied in wash 2 are listed in table 2.

Fractions were collected in UV-readable 96 well plates and UV absorbance was read at 280 nm. Pseudo-chromatograms of the parallel chromatography run are shown in figure 2. Guanidinium hydrochloride washing and arginine washing lead to elution of UV 280 nm absorbing material.

The different solutions applied in wash 2 are indicated in the legend. Guanidinium hydrochloride and arginine induce elution of UV 280 nm absorbing components during post-load washing. The corresponding elution peaks are smaller.

PSEUDO-CHROMATOGRAMS OF TOYOPEARL AF-rProtein L-650F FOR CAPTURING OF SCFV FROM E.COLI

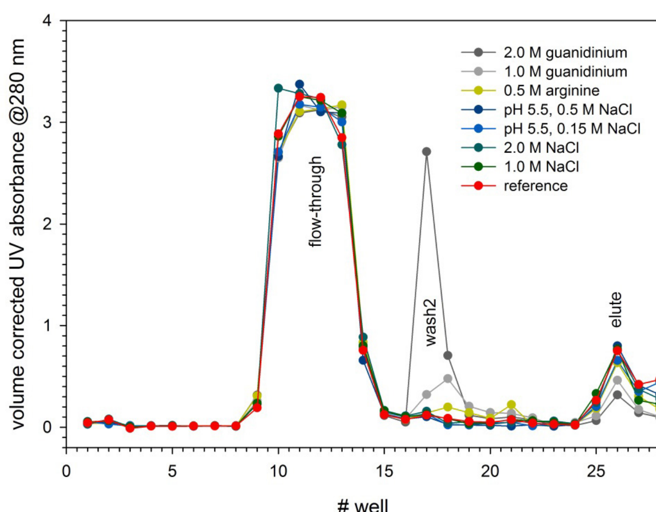


Figure 2

NORMALIZED SEC CHROMATOGRAMS OF THE PARALLEL CHROMATOGRAPHY ELUTION FRACTIONS AND FEEDSTREAM

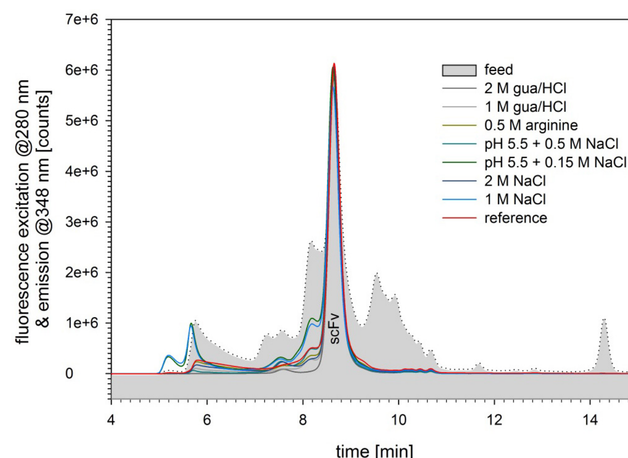


Figure 3

OFF-LINE ANALYSIS

The different fractions of one process step were pooled for off-line evaluation. The subsequent analysis included SEC using TSKgel® G2000SWxl 7.8 mm ID x 30 cm L (P/N 0008540), a Protein L leaching ELISA and an ELISA assay for the determination of HCP from E.coli. For SEC, a 100 mM sodium phosphate buffer, pH 6.7 was complemented with 100 mM sodium sulfate. 25 µl aliquots of the process steps were injected and separated at 1.0 mL/min. SEC chromatograms are shown in figure 3. The scFv elutes at 8.4 min. Guanidinium hydrochloride washings lead to highest scFv purity.

Guanidinium hydrochloride and arginine washings improve scFv purity. The majority of the impurities visible in SEC elute directly upfront of the product, suggesting these peaks may represent misfolded an aggregated scFv. Guanidinium hydrochloride washings may lead to on-column refolding and depletion of less specifically bound misfolded scFv.

LOG REDUCTION VALUES FOR HCP REMOVAL DURING PROTEIN L CHROMATOGRAPHY

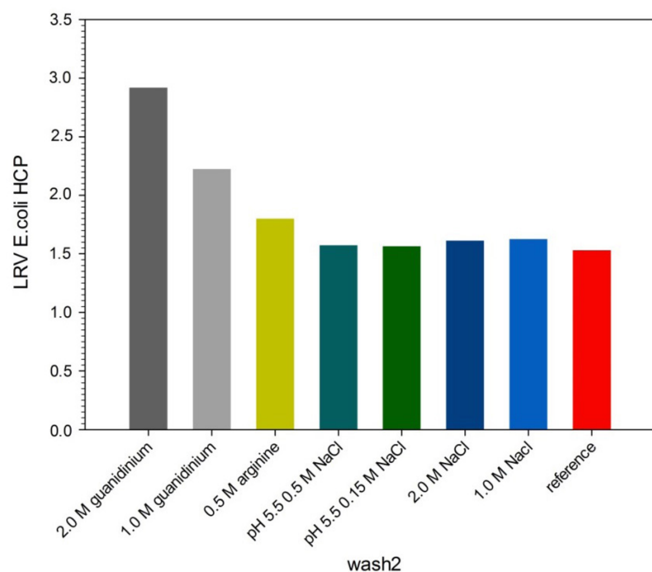


Figure 4

All elution pools contain less than 20 ng leached Protein L per mL.

CONCENTRATION OF LEACHED PROTEIN L IN THE ELUTION POOLS OF THE PARALLEL CHROMATOGRAPHY EXPERIMENT

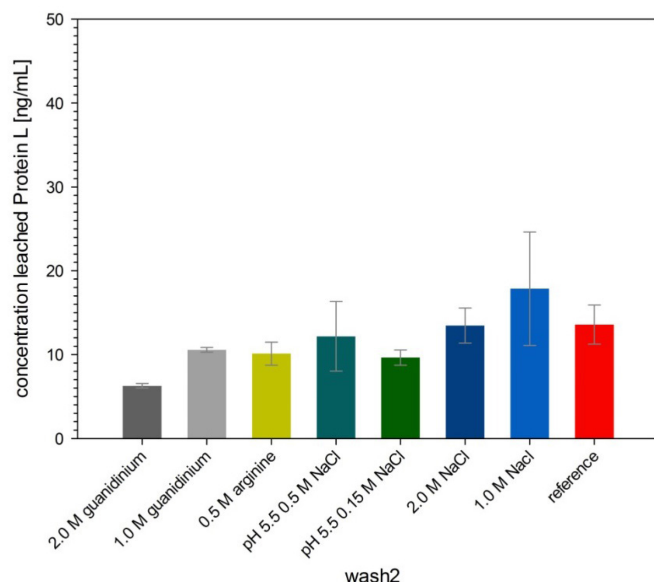


Figure 5

HCP removal is more than 10 x higher if 2 M guanidinium hydrochloride is applied during post-load wash compared to the equilibration buffer wash, which served as a reference in this study.

Further, the guanidinium and arginine washed elute pools show lower HCP burden (Figure 4). Washing with 2 M guanidinium hydrochloride increases the LRV for HCP from 1.6 to 2.9. The tested conditions did not lead to enhanced Protein L ligand leaching, as shown in figure 5.

CONCLUSION

According to the herein presented results, post-load washing with chaotropic agents can significantly improve HCP removal during capturing of a scFv from E.coli using TOYOPEARL AF-rProtein L-650F. It may further support on-column refolding of misfolded and aggregated product.

ACKNOWLEDGEMENTS

The scFv feedstream produced in *E.coli* was kindly provided by Dr. Oliver Seifert, Institute of Cell Biology and Immunology, University of Stuttgart.

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